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Estrogens in maternal plasma following intraamniotic injection of (^{3}H)-dehydroepiandrosterone-sulfate in midpregnancy

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This investigation was initiated to study the kinetics of C_{19} and C_{18} steroids in maternal plasma following application of ^{3}H-labelled estrogen precursors to the fetal compartment. It was hoped to obtain information about the quantity and quality of the fetoplacental steroid-metabolism in the direction of fetus to mother.

In the past years LAURITZEN and coworkers [5, 6, 7] have developed a placental function test in which dehydroepiandrosterone-sulfate as an estrogen-precursor was injected into the mother. Thereafter the increase of the estrogen-excretion in the 24-h-urine or the estrogen level in maternal plasma was determined as a parameter of placental metabolic activity [20]. Both urinary and plasma estrogens rose markedly after application of the androgenic steroidprecursor as confirmed by investigators [2, 21, 19].

The DHEA-S induced increase in urinary or plasma estrogens provides information about the reserve-capacity of the placenta. Using incubation studies with placental tissue we could demonstrate in vitro that the conversion of DHEA to estrogens by the placental aromatising enzyme-system is significantly reduced in pathological pregnancies (pre-eclampsia, diabetes, postmaturity) as compared to normal pregnancies [10, 12, 13]. These studies demonstrated that in complicated pregnancies the diminished capacity of the aromatizing enzyme-system can occur and may result in a reduced estrogen production which is of great clinical importance. Until now the dehydroepiandrosterone-test was performed by precursor application to the maternal compartment i.e. by intravenous injection of DHEA-S into the maternal cubital vein. Since the amniotic fluid has become more easily accessible due to better techniques the possibility of a new placenta function test using intraamniotic DHEA-S injection was examined simultaneously. In the following investigations of 4 normal pregnancies between the 18th to 20th week of gestation, we applied (^{3}H)-labelled dehydroepiandrosterone-sulfate directly into the fetal compartment i.e. intraamniotical. Subsequently these pregnancies were legally terminated by injection of prostaglandine after the test-procedure.

1 Materials and methods

After locating the placenta by ultrasonic vision in 4 patients, 100 μCi DHEA-7α-^{3}H-sulfate (spez. activity 1000 mCi/mmol) + 5 mg DHEA in sterile 0.9% NaCl were injected transabdominally into the amniotic cavity. Ten milliliters of blood was drawn from the cubital vein of the pregnant women before injection and after 15, 30, 60, 120, 180 und 240 minutes into heparinized tubes. After centrifugation 5 ml of plasma was incubated with 5000 IU β-glucuronidase sulfatase (BOEHRINGER) for 2 hrs. at pH 5.4 and 37°C in a shaking thermostat. Extraction of the total free metabolites was processed three times with ether/chloroform.

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The extracts were evaporated to dryness and the dry residues were redissolved in warm methanol and pipetted into formamid-saturated paper for chromatography. Separation of C_{18} and C_{19}-steroids was performed in formamid/monochlorbenzene.

The C_{18}-steroids were rechromatographed in formamid/chloroform, the C_{19}-steroids in propylenglycol/cyclohexan (see KNUPPEN [4] for further details). The resulting metabolites estrone (Oe_1), 16α-hydroxyestrone (16α-OH-Oe_1) and estradiol-17β (OE_2-17β) as well as the androgens, Δ_4-androstenedione and testosterone were reduced or oxidized, rechromatographed and characterized in the following manner:

1. Oxidation by 3, 17β-hydroxysteroid-reductase (EC 1.1.1.51) type I *Sigma Chemicals*, St. Louis, USA.
2. Reduction by sodiumboranate in methanol.

Estriol (Oe_3), DHEA and 16α-OH-DHEA were partially acetylated and identified by recrystallization to constant specific activity.

After localization of the metabolites by parallel-running, labelled test-chromatogramms, extraction from the paper was performed with methanol and radioactivity measured in a liquid-szintillation-counter. The quantity of plasma-steroids was expressed in nCi/100 ml plasma.

2 Results

The course of the plasma-levels for each steroid-fraction during the test-period is shown in Tab. I. The values for the 4 isolated C_{18}-steroids over the test-period are additionally demonstrated in graphs (see figures). From the C_{18}-steroids, the two C_{16} hydroxylated compounds, estriol and 16-hydroxyestrone, have been isolated. Seventy-five percent of all originating estrogens were hydroxylated at C_{16}. Estradiol-17β and estrone were found in approximately the same quantity. In contrast the estriol level was 2 to 3 times higher than that of the other estrogens. The peak plasma concentration of all C_{18}-steroids appeared 180 minutes after (^{3}H)-DHEA-S injection with the exception of estrone which reached its maximum concentration slightly earlier, that is after 120 minutes.

From the C_{19}-steroid-fraction, 16α-hydroxy-DHEA was isolated with highest concentration in plasma. The recovery of unchanged (^{3}H)-DHEA-S, the steroid that had been initially injected, was somewhat lower.

The plasma-levels of Δ_4-androstenedione and testosterone were almost equal. The plasma-levels of the C_{19}-steroids showed the same temporal course as those of the C_{18}-steroids. The highest values were detected at 180 minutes.

3 Discussion

The fetal adrenal cortex produces 75% of the total DHEA-S which is then primarily C-16-hydroxylated in the fetal liver. DHEA-S and 16α-OH-DHEA-S reach the placenta as estrogen-precursors through the umbilical arteries and are converted via androstenedione and 19-hydroxyandrostenedione to estrogens, mainly to estriol [1, 14, 17, 18]. For this conversion the placenta is provided with the following enzymes arylsulfatase, 3β-hydroxysteroiddehydrogenase-Δ^4-Δ^5-isomerase, 19-hydroxylase and the aromatizing enzymesystem. The additional activity of the placental microsomal enzyme 17β-hydroxysteroiddehydrogenase causes formation of testosterone by reduction of androstenedione and also of estradiol-17β from estrone [16, 8]. In our injection study we simulated physiological processes described above by applying the labelled and unlabelled DHEA directly to the feto-placental unit by intraamniotical injection.

The quantity of the exogenous hormones administered in our test is within the normal physiological range. The normal fetus produces about 70 mg DHEA-S in 24 hrs.; however, we injected 5 mg unlabelled DHEA and measured its metabolites over a 4 hr. period. Only 5 μg of labelled DHEA was used to enable qualitative analysis.

Previous investigators have shown that prior to the 20th week of gestation the fetus swallows significant volumes of amniotic fluid. The high 16α-hydroxylaseactivity of the fetal liver [9, 11] causes C_{16}-hydroxylation of 75% of all recovered metabolites (Tab. I): The main 16-hydroxylated metabolite is 16α-OH-DHEA and after placenta-passage 16α-OH-estrone and estriol. 15 minutes after intraamniotic injection of DHEA-S a signif-

Tab. I. Level of plasma estrogens (nCi/100 ml plasma) and of androgenic precursors following intraamniotic injection of 100 μCi (^{3}H)-dehydroepiandrosterone-sulfate.

Time (min)	Oe_3	Oe_2-17β	Oe_1	16α-OH-Oe_1	DHEA	16α-OH-DHEA	Δ_4-Androst.	Testosteron
				Case 1.				
15	260	63	48	41	63	75	24	32
30	340	91	100	60	78	102	48	52
60	490	130	190	70	97	120	65	67
120	520	150	200	85	100	125	79	98
180	710	262	240	100	82	140	85	100
240	640	220	203	90	60	79	43	91
				Case 2.				
15	325	75	65	49	75	90	29	38
30	410	110	124	72	95	122	50	63
60	580	156	230	84	120	134	78	89
120	650	180	270	102	131	151	95	112
180	810	324	293	121	128	168	101	121
240	720	268	254	110	75	96	52	99
				Case 3.				
15	340	100	90	51	90	121	34	44
30	430	110	130	71	102	142	52	63
60	600	170	200	85	112	154	69	74
120	675	200	250	90	140	160	85	90
180	870	305	170	105	120	187	97	80
240	750	270	100	80	82	136	66	56
				Case 4.				
15	240	72	81	68	75	101	28	37
30	325	89	122	79	98	112	38	49
60	456	120	154	85	110	134	49	62
120	528	172	163	108	130	158	59	78
180	690	218	134	145	122	177	85	72
240	581	154	103	110	91	127	44	41

icant quantity of labelled metabolites appears in the maternal circulation. As illustrated in the graphs, the plasmalevels of the metabolites in all 4 cases tested increased up to 3 hrs. after injection and then decreased rather quickly. Our interpretation of the results is, that the fetus continously swallows the amniotic fluid (approx. 300–500 ml in the 20th week fo gestation) containing the dispersed radioactive steroid, metabolizes it and delivers it into the maternal circulation after placental passage. This consecutive release of steroid hormones by the placenta might explain the relatively high levels in maternal plasma over several hours, since the elimination of steroids by the kidney is rapid i.e. within 30 minutes. Twenty five percent of the swallowed DHEA is not C_{16}-hydroxylated and therefore seems not to pass the fetal liver and consequently to reach the placenta without 16α-hydroxylation. Here it is converted to Δ_4-androstenedionetestosterone and to estrone and estradiol-17β via the Δ_4-metabolic pathway. These fractions enter the maternal circulation and were identified in plasma as shown in table I. One could assume that a small part of the intraamniotically injected DHEA could be reabsorbed by the amnion and reaches the maternal circulation

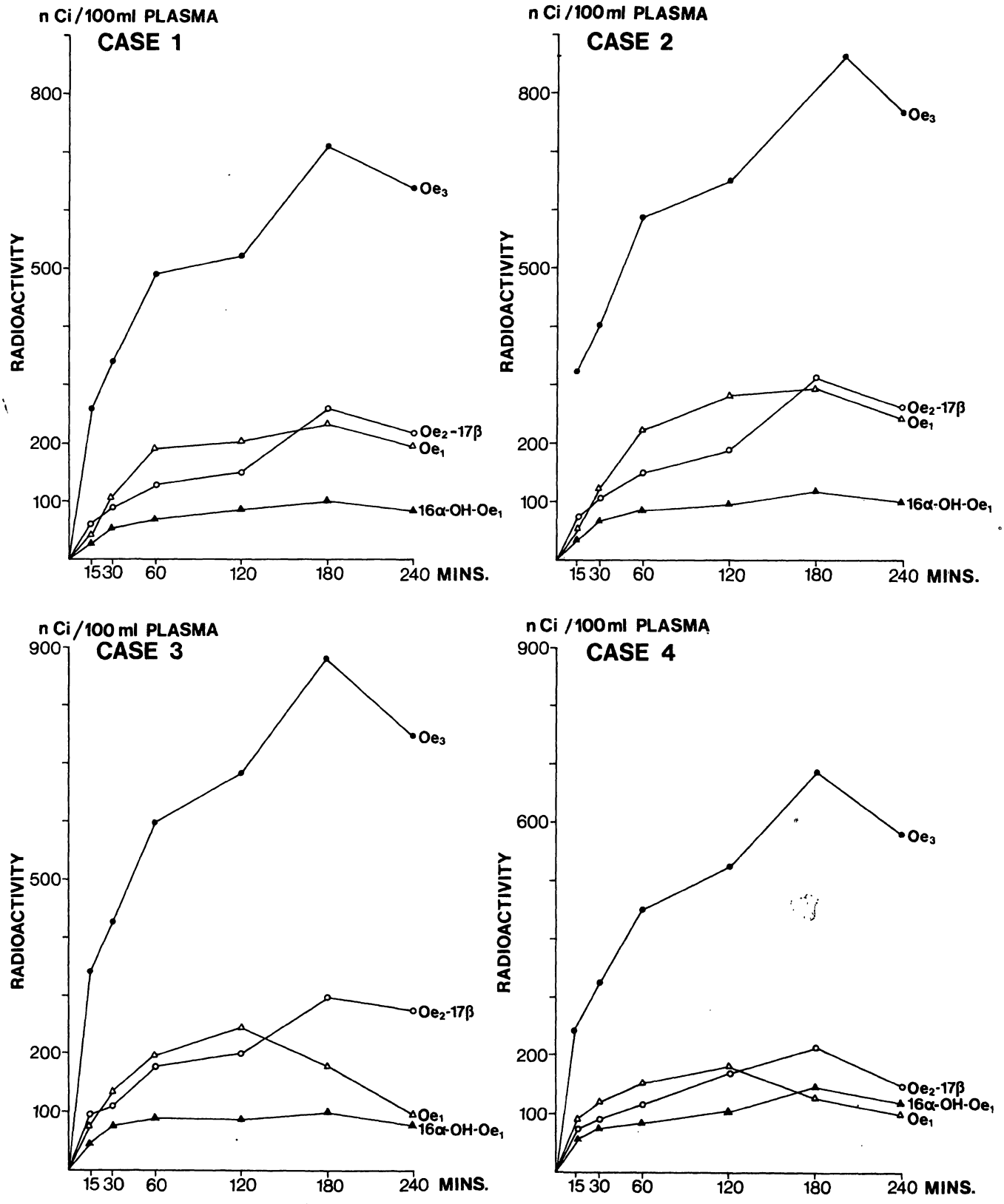

Fig. 1–4. Levels of the four main C_{18}-steroids in maternal plasma of 4 patients following intraamniotic injection of 100μ/Ci DHEA-7α-^{3}H-sulfate in midpregnancy.

directly in this manner without 16α-hydroxylation. This assumption is supported by the recovery of small amounts of unmetabolized labelled DHEA.

HAUSKNECHT and MANDELMAN [3] have found an increase of estriol in 24-hr-urine samples after intraamniotic application of unlabelled DHEA. Other metabolites were not isolated. Similar investigations were carried out by MICHIE [15] in pregnancies with anencephalic fetuses. In these cases the estriol excretion in 24-hr-urine was significantly lower than in normal pregnancies. Our study shows that the application of labelled estrogen-precursors to the fetal compartment and the following recovery of C_{19}- and C_{18}-steroids in maternal plasma give valuable information about the quantity of the fetoplacental steroid-metabolism in midpregnancy.

Summary

In 4 patients with normal pregnancies between the 18th and 20th week of gestation (^{3}H7α)-dehydroepiandrosterone-sulfate ([^{3}H]-DHEA-S) was injected intraamniotically. Maternal venous blood was drawn before and at regular intervals for 240 minutes after DHEA-injection. Thereafter, legal abortion was performed by intraamniotic instillation of prostaglandine. The conjugated steroids were hydrolyzed enzymatically and the total steroids were isolated and identified.

The following labelled metabolites were determined quantitativeley:
Estriol (E_3), estradiol-17β (E_2-17β), estrone (E_1), 16α-hydroxy-estrone, (16α-OH-E_1), dehydroepiandrosterone (16α-OH-DHEA), Δ_4-androstenedione (AD) and testosterone (T).
The maximal increase of all estrogen fractions in maternal plasma occurred 120–180 min after intraamniotic injection of the precursor. The most prominent rise of the C_{18}-steroids could be shown for estriol. 60–70% of all metabolites were C_{16}-hydroxylated.

Keywords: Feto-maternal steroid metabolism, intraamniotic injection of dehydroepiandrosterone.

Zusammenfassung

Östrogenspiegel im mütterlichen Plasma nach intraamnialer Injektion von ^{3}H-Dehydroepiandrosteron-Sulfat in der Mitte der Schwangerschaft.
(^{3}H)-Dehydroepiandrosteron-Sulfat (DHEA-S) wurde bei vier normalen schwangeren Frauen in der 18.–20. Gestationswoche direkt intraamnial verabreicht. Die Schwangerschaften wurden später legal durch Prostaglandin-Instillation unterbrochen. 15 bis 240 min nach der Injektion wurde Blut aus der mütterlichen Kubitalvene entnommen. Im Plasma erfolgte die enzymatische Hydrolyse, die gesamten Steroide wurden isoliert und identifiziert. Folgende radioaktive Metabolite wurden schließlich quantitativ gemessen: Östriol (Oe_3), Östradiol-17β (Oe_2-17β), Östron (Oe_1), 16α-OH-Östron, DHEA, 16α-OH-DHea, Δ_4-Androstendion und Testosteron. **Der maximale Anstieg aller Östrogene erfolgte im Plasma 120–180 min nach Injektion des Vorläufers. Die Östriolfraktion überwog bei weitem die der anderen C_{18}-Steroide.** 60–70% der Metabolite insgesamt sind an C_{16} hydroxyliert. Abschließend kann auf Grund unserer Ergebnisse festgestellt werden, daß durch intraamniale Verabfolgung markierter Östrogenvorläufer und durch die Wiederfindung der C_{19}- und C_{18}-Steroide im Plasma der Mutter eine gute Auskunft über die Quantität und Qualität des fetoplacentaren Steroidstoffwechsels erhalten werden kann.

Schlüsselwörter: Foeto-materneller Steroidstoffwechsel, intraamniale Injektion von Dehydroepiandrosteron.

Résumé

Les oestrogènes dans le plasma maternel résultant d'une injection intraamniotique de (^{3}H)-déshydroépiandrostéronesulfate en milieu de grossesse
Une injection intraamniotique, c.à.d. par le côté foetal, de (^{3}H7α)-déshydroépiandrostéerone-sulfate ([^{3}H]-DHEA-S) a été faite chez 4 parturientes à la grossesse normale entre la 18ème et 20ème semaine de gestation. Les grossesses ont été interrompues légalement par la suite par instillation intraamniotique de prostaglandine. Du sang a été prélevé dans la veine cubitale de la mère à intervalles réguliers avant et jusqu'à 240 min après l'injection. L'hydrolyse enzymatique s'ensuivit dans le plasma, tous les stéroides furent isolés et identifiés. Enfin on a mesuré quantitativement les métabolites radioactifs:
oestriol (E_3), oestradiol-17β (E_2-17β), oestrone (E_1), 16α-hydroxy-oestrone, (16α-OH-E_1), déshydroépiandrostérone (DHEA), 16α-hydroxy-déshydroépiandrostérone, 16α-OH-DHEA), Δ_4-androsténédione (AD) et testostérone (T).
L'augmentation maximale de tous les oestrogènes survint dans le plasma 120–180 min après l'injection du précurseur. La fraction d'oestriol dépassa de beaucoup la

hausse des autres stéroides C_{18}. 60–70% de tous les métabolites sont hydroxylés à C_{16}. En conclusion on peut constater à la suite de nos résultats qu'il est possible d'obtenir de bonnes indications sur la quantité et la qualité du métabolisme stéroide foeto-placentaire en administrant des précurseurs oestrogéniques marqués de façon physiologique par le côté foetal et en retrouvant les stéroïdes C_{19} et C_{18} dans le plasma de la mère.

Mots-clés: Injection intraamniotique de déshydroépiandrostérone, métabolisme stéroïde foeto-maternel

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